Original Article

Estimation of Antibodies against Saccharomyces Cerevisiae in Patients with Indeterminate Colitis

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Summary:

Background: Indeterminate colitis (IC) originally referred to those 10-15% of cases of inflammatory bowel disease (IBD) in which there was difficulty distinguishing between ulcerative colitis (UC) and Crohn's disease (CD) in the colectomy specimen and histopathology examination. However, IC is increasingly used when a definitive diagnosis of UC or CD cannot be made at colonoscopy examination, colonic biopsies or at colectomy. The diagnostic difficulties may explain the variably reported prevalence of IC. Clinically, most patients with iC evolve to a definite diagnosis of UC or CD on follow up.

Patients and methods: PATIENTS GROUP: Consisted of 80 patients with indeterminate colitis (IC), their age ranged (16-84 years), mean age was 45.5 years, 50 (62.5%) were males and 30 (37.5%) were females. CONTROL GROUP: Consist of 40 healthy volunteers, mean age was 39.3 years, 20 of them were male and the rest were females. Anti-Saccharomyces cerevisiae antibodies (ASCA) were detected using indirect immunofluorescence test (IIF) test.

Results: Results of ASCA antibodies in the serum of IC patients showed significant higher frequency (66.2%)(p>0.001) than control group(7.5%). The commonest isotype of this ASCA antibody was IgG (53.7%) that is significantly higher than control group (P>0.001). The sensitivity of ASCA was 63.1 %, specificity was 28.5% and positive predictive value of ASCA was 80% and negative predictive value was 78.5%.

Conclusions: In conclusion, higher frequency of ASCA (IgG) expression in IC was useful in early estimation of IC and could be used as serological marker.

Keywords: Anti Saccharomyces Cerevisiae Antibody (ASCA), indeterminate colitis, immuno fluorescence.

Fac Med Baghdad 2010; Vol. 52, No. 1
Received Sep.2009
Accepted Nov. 2009

Introduction:

In 1978, Price introduced the term indeterminate colitis (IC) to refer to a subgroup of approximately 10-15% of inflammatory bowel diseases (IBD) cases in which there was difficulty in distinguishing between UC and CD in the excised colectomy specimen(1). The inflammatory bowel diseases (IBDs), Crohn's disease (CD) and ulcerative colitis (UC), are a heterogeneous group of disorders of unknown etiology (2). Accurate classification systems and precise diagnostic tools are needed. This is not only important for prognosis and treatment but also to better understand the pathogenesis of IBD. IC was considered a temporary classification until a final diagnosis was established during follow-up. Over time, the concept of IC has evolved. Because of the widespread and growing use of endoscopy and biopsy (3), the diagnosis of IC in most cases were based on microscopic histopathology and clinical features when no diagnostic features of either CD or UC can be found(4). The diagnosis is often inconclusive. It should be recognized that these patients might represent a specific subgroup of IBD (5). Indeed, the presence of IC supports the concept that IBD Represents a spectrum of diseases rather than just 2 entities, CD and UC.Antibodies to several specific antigens had been reported in the sera of patients with IBD. It was hoped that studies of such antibodies would provide either insight into disease pathogenesis and heterogeneity or putative serological markers to adjunct/replace current diagnostic protocols. Great interest has been shown in anti-Saccharomyces cerevisiae antibodies (ASCAs), associated first with CD in the 1980s (6). These antibodies have 60-70% prevalence in CD patients compared with 10-15% in UC and 0-5% in healthy control subjects (7). Since 1988, ASCAs recognition is associated with CD. An antibody that targets the phosphopeptidomannan part of the cell wall of S cerevisiae (ie, Baker's yeast), ASCAs have consistently been found in 50% to 80% of CD patients (8). Although little is known about the origin of this serologic marker and consequently about their potential value in IC, this marker have been proposed to aid diagnostic accuracy. ASCA was noninvasive and may be helpful in the identification of subgroups of patients with IC. The possible usefulness of ASCA in patients with IC has been considered previously (9). The aim of this study was to estimate the frequency of ASCA in IC patients, isotype and titer. The other goal was to determine the sensitivity and specificity of this serological test.

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Patients and methods:

PATIENTS GROUP: Consisted of 80 patients with indeterminate colitis (IC), their age ranged (16-84 years) , mean age was 45.5 years, 50 (62.5%) were males and 30 (37.5%) were female. 45 (56.2%) were current smokers. Most of them were complaining from abdominal pain, bleeding per rectum, constipation alternate with diarrhea and tensmus. Patients with IC were those whose initial diagnosis of IBD could not be confirmed clearly as CD or UC. They defined as indeterminate colitis (IC) by their specialist physicians according to the clinical, endoscope (figure-3,4), radiologic and histopathological examinations (figure-2-). They were admitted to Al-Kindi Teaching Hospital - Colonoscopic department from September -2008 to May- 2009. 95% of them from Baghdad and the rest from other provinces. Infectious colitis and other form of colitis were excluded by stool culture and parasitic examinations. The other group was CONTROL GROUP: Consist of 40 healthy volunteers, mean age was 39.3 years, 20 of them were males and the rest were females. Blood samples were collected from the two groups and serum were collected from same individuals and stored at -10C0 till examination was done in the microbiology and immunology laboratory in Al-Kindi College of Medicine - Baghdad University, Diabetic and endocrinology Center-Immunological laboratory. Indirect immunofluorescence test (IIF): Anti-Saccharomyces cerevisiae antibodies (ASCA) were detected using IIF test. To prepare slides, S cerevisiae (dried yeast, Be-Ro) was cultured on Sabouraud agar (Oxoid) for 48 hours at 37C0. The growth was harvested, washed and resuspended in 0.15M sterile saline. The suspension was smeared on Teflon coated slides and fixed with 95% ethanol after drying and stored at -10C0 till used (10), briefly, samples were initially diluted to 1:10 in phosphate buffered saline. An FITC conjugated rabbit antihuman (mixed IgG IgM IgA) antibody (Dako, Copenhagen, Denmark) was used for detection of bound immunoglobulin ,then isotype was determined by using FITC conjugated rabbit antihuman IgG, FITC conjugated rabbit antihuman IgM, and FITC conjugated rabbit antihuman IgA antibodies. The positive serum (1:10) was further diluted to 1:100 and 1:1000 and all the above procedure was repeated using different isotypes. All slides were evaluated by two independent observers; in the event of a difference in opinion, a third observer was decisive. Statistical analysis: Student's t-test used in analysis of the data statistically. Results were expressed as percentages. In addition to that, Sensitivity was the probability of a positive ASCA in a patient with IC; specificity was the probability of a negative ASCA in a patient with IC. The positive predictive value (PPV) was the probability of having CD in a positive ASCA; the negative predictive value (NPV) was the probability of having UC in a negative ASCA were also computed (11).

Results:

Eighty patients with IC were included in this study. Fifty of them (62.5%) were males and the rest were females. Their age ranged (16-84 years). There was a significant difference in age at sampling between patients group and control group (P>0.001).

About 56.2% of them were current smokers that are not significantly different from the control group (Table-1-).

Table-1- Demographic data for IC patients and healthy control.

	Indeterminate colitis No.=80 No. %		Healthy control No.=40 No. %		P value
Sex Male female	50 30	62.5 37.5	20 20	50 50	
Age at sampling X ± SD	45.5±26.4		39.3±42.5		P>0.001
Age range	16-84		17-65		
Smoking status					
Never	10	12.5	15	37.5	P<0.05
Current	45	56.2	20	50	NS
Ex-smoker	24	31.2	5	12.5	P<0.05

NS= not significant

Results of frequency of ASCA + antibodies (figure-1-) in the serum of IC patients showed significant higher frequency (66.2%)(p>0.001) than control group(7.5%) (Table-2-). The sensitivity of ASCA was 63.1 %, specificity was28.5% and predictive value of ASCA were shown in table-2-.

Table-2- Results of frequency of ASCA in IC patients compared with control group with (sensitivity, specificity, positive and negative predictive value).

frequency of ASCA	Indeterminate colitis No.=80 No. %		Healthy control No.=40 No. %		P value
ASCA (+)	53	66.2	3	7.5	P>0.001
ASCA(-)	27	33.7	37	92.5	P>0.001

	sensitivity	specificity	Positive predictive value	Negative Predictive value
ASCA+	63.1%	28.5%	80%	78.5%

The commonest isotype of this ASCA antibody was IgG(53.7%) that is significantly higher than control group(P>0.001) (Table-3). The highest titer of IgG type was 1:1000 in 22/43 of positive IgG isotype (51.1%) which is significantly higher than control group (p>0.001) as shown in table-3-.

Table-3 - ASCA isotype and titer in IC patients and control group.

ASCA	Indeterminate colitis No.=80 No. %	Healthy control No.=40 No. %	P value	Indeterminate colitis 1:10 No. %	Indeterminate colitis 1:100 No. %	Indeterminate colitis 1:1000 No %	Healthy control 1:10 No. %
ASCA mix (IgG,IgM,IgA)	53 66.2	3 7.5	P>0.001				
ASCA(IgG)	43 53.7	3 7.5	P>0.001	43 53.7 P>0.001	21 48.8 P>0.001	22 51.1 P>0.001	3 7.5
ASCA(IgM)	16 20	1 2.5	P>0.001	16 20 P>0.001	4 25 P>0.001	0 0 NS	1 2.5
ASCA(IgA)	1 1.2	0 0	NS	1 1.2 NS	0 0	0 0	0 0

NS=not significant

There was no significant difference of ASCA level with site of the lesion in colon (table-4-).

Table-4-Results of ASCA with the lesion site in the colon.

Site of lesion	ASCA+ No	%	ASCA- No	%	P value
Rectum	11	20	4	14.8	NS
Sigmoid colon	8	15	2	7.4	NS
Ascending colon	13	24	4	14.8	NS
Transverse colon	2	3.7	2	7.4	NS
Descending colon	0	0	0	0	NS

NS=not significant

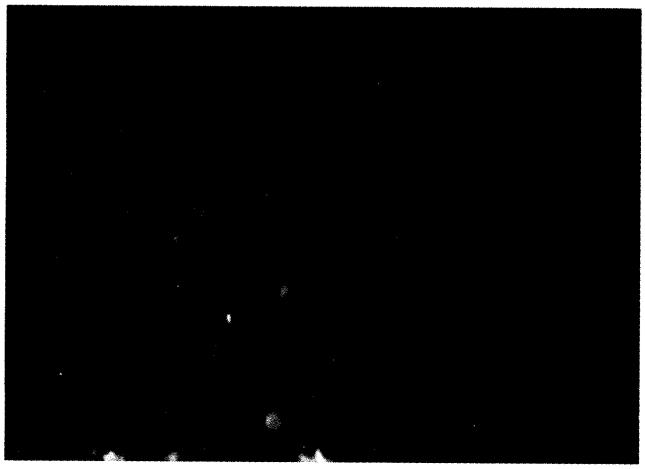

Figure-1- Antibodies against Saccharomyces cerevisiae by indirect immunofluorescence test

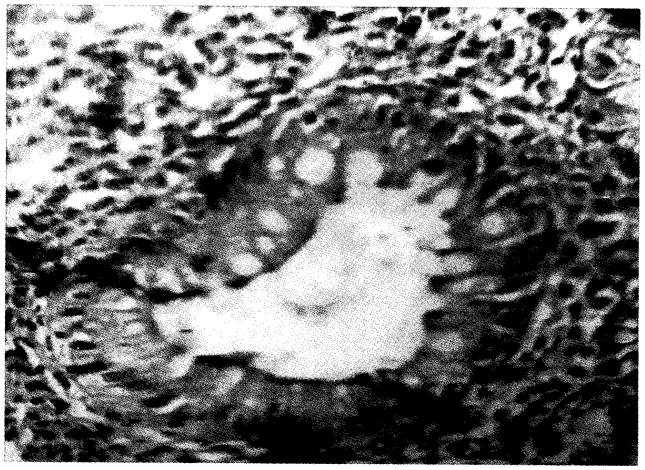

Figure-2- histopayhological examination showed infiltration with chronic inflammatory cells

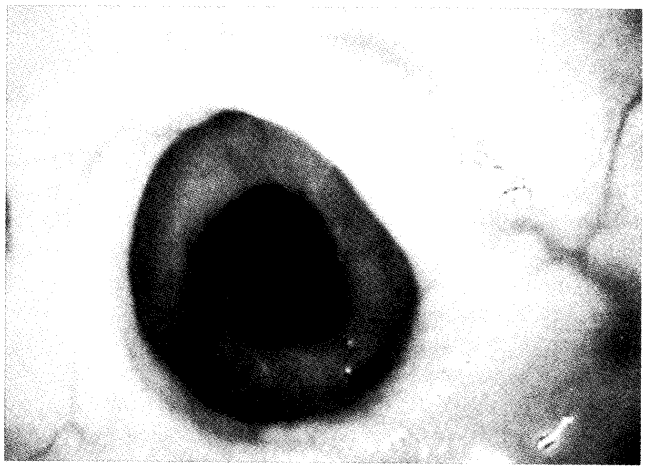

Figure-3- edematous mucosa with lose of normal vascular pattern.

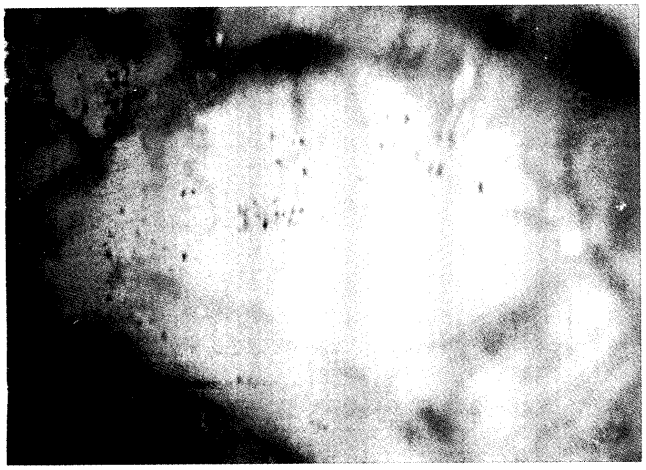

Figure-4- colonic mucosa showed ulceration.

Discussion:

In most cases of IBD, a differentiation between CD and UC can be achieved with conventional diagnostic methods. However, an exact diagnosis cannot be made in approximately 10%-15% of patients with colitis, and these patients are classified as having IC. Despite the fact that IC is a rather temporary diagnosis and that up to 80% of patients with this designation will be diagnosed with CD or UC over time (12). Although the etiology is unknown, it is increasingly clear that this disease represent the outcome of three essential interactive cofactors: environmental factors (for example, enteric microflora), multigenic host susceptibility, and immune mediated tissue injury. A variety of immune abnormalities have been described in IC, both at the systemic and intestinal levels, several autoantibodies differentially associated with IC have been investigated in this respect. Strong antibody responses to a variety of epitopes have been documented. The antibodies that have been most thoroughly studied are antibodies against mannose epitopes from the yeast Saccharomyces cerevisiae (ASCA)(13). In our study, 66.2% of IC developed ASCA, that was in agreement with other study that showed (61.5%) in IC patients had ASCA+ (14). Several studies have been promoting ASCA as a noninvasive diagnostic tool in the gastroenterologist's practice. However, before a diagnostic test can be used in clinical practice, both a high sensitivity and specificity for the test are needed. If the aim is screening subjects at risk for IC, a high sensitivity is of great importance. If the test is used for differentiating between phenotypes, high specificity is necessary. Thus, sensitivity of our study was 63.1%which was in accordance with Joossens etal 2002 (66.7%) and the specificity was 28.5% . Other authors like Mokrowiecka etal 2007(15) found sensitivity of ASCA in IC was 72% and specificity was 63%. In case of PPV in our study was 80% and NPV was 78.5%, this means that ASCA + predicts evolution to CD in 80% of the patients, ASCA- predicts UC in 78.5% of patients. This was in agreement with other study that PPV was 80% and NPV was 63.6 %(14). Moreover, the prognosis in patients with IC is worse because there was a higher frequency of relapse and an increased risk of colon cancer (16). The reason for generation of ASCA remains unclear. it had been found that ASCA react with sequences of mannose residues expressed in the cell wall mannan of S cerevisiae(17). It was hypothesised that increased permeability in the small bowel of IC patients might lead to increased exposure of yeast antigens (which are a resident part of the normal intestinal flora) to immune reactive cells. Increased permeability of the small bowel as an early event in the pathogenesis of IC (before gross damage to the bowel wall is apparent) may also explain our findings of the early appearance of ASCA. The isotype of ASCA in our study was IgG (53.7%) and IgM was the second one (20%) and no ASCA IgA results were found .This indicated the early formation of (IgM) (primary immune response) and later on , the second exposure to the same antigen leads to formation of IgG isotype. Several studies had been found ASCA expression (either IgA or IgG) (18). The highest titer 1:1000 of IgG (51.1%) was found in patients with IC .this higher titer of IgG increased risk of surgery (19).Therefore; ASCA might be used as early prediction of microscopic colitis (20) and beneficial in the management of IC (21). In the case of site, we found higher percentage of ASCA+ (24%) in patients who had lesions in ascending colon and rectum (20%) and less in other sites of colon. Finally, ASCA could be considered as a serological marker in IC that in agreement with other results (22).

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